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Growth response of pre-sprouted seedlings of sugarcane in the presence of the bacterium *Herbarspirillum frisingense*

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***Abstract*— *The objective was to evaluate the effect of bacterium Herbaspirillum frisingense on growth of the seedlings pre-sprouted from sugarcane. After you select the varieties of sugarcane, the necessary treatments of nodal segment obtained from reeds, to germinate and be transplanted to the cells half in soil substrate of gully and other half in soil ofgullies more Jatropha pie, where was subsequently applied doses of bacteria (9,33x10^7 ufc.ml^{-1}). Five months after this process, plants were harvested and evaluated the dry mass of shoot and root dry mass. Data were analyzed by Tukey, a 5%, where he met difference for root dry mass in relation to the presence of bacteria and to dry pasta from the shoot to the type of substrate. No statistical difference was observed between cultivars, even these being different cycles. In relation to interaction varieties vs. bacteria statistical difference was observed between the cultivars to root dry mass. The presence of the bacteria inside the plant tissues of seedlings of sugarcane was beneficial for the plant, promoting a greater root growth. The soil substrate of gully more Jatropha pie turned out better for the cultivars in general.*

***Keywords*— Endophytic bacteria; seedling production; Saccharum officinarum L.**

I. INTRODUCTION

The sugarcane (*Saccharum officinarum*), culture introduced in Brazil in the 16th century, has great economic importance in the country, according to cultivable areas and ideal soil and climate conditions the production, making the country a participant in World marketing with high competitiveness [1, 2].

According to the National Supply Company, Brazil is the largest producer of sugar cane with a productivity expected to yield the 2018/19 of 66.007 kg/ha with a harvested area of 30, 3 thousand hectares and a total estimated production 625, 96 million tons, much of this production will be destined to the production of ethanol is expected to reach a total of 154, 96 million liters [2].

It is known that much of the production is concentrated on the South and Midwest of the country, however, in the North, the Tocantins is regarded as the new agricultural frontier, offering areas, weather, and water conditions. The State owns one of the main natural elements that favor the productivity of sugarcane, the luminosity, since, under the conditions of the State climate factor that tends to increase the number of profiling [3].

The system of seedlings pre-sprouted (SPS), or results pre-germinated in a correct planting, with healthy seedlings and that there is no competition between plants online, resulting in better initial growth. The technique can be used by all producers, small medium and large, for this is a simple, low-cost methodology allowing the planting of reestablished seedlings, and avoiding competition between sounds plants [4].

To increase the productivity, are being carried out studies and research of bacteria capable of contributing to a better synthesis initial growth of seedlings pre-germinated. Endophytic bacteria have numerous functions for the plant, acting beneficial and showing satisfactory results and significant. Bacteria that inhabit the interior of the plant tissue can contribute effectively to the biological fixation of nitrogen, since the exchange is done directly, having this way a lesser competition for carbon sources, noting that not all micro-organisms are able to penetrate plant tissue[5].

Bacteria of the genus Herbaspirillum are described as

diazotrophic, able to fix nitrogen from the atmosphere under microaróbicas conditions and use of this resource to meet all your demand for this nutrient when compared to the other species of diazotrophic bacteria these are the least of it. Representatives of this genus are considered compulsory and feature low endophytic bacteria survival in soil [5, 6].

Was used to perform this experiment to frisingense of the same genre, which behaves similar to H. seropedicae. For this are already found numerous studies because it is a bacterium that is associated with various agricultural plants and promotes your growth H. frisingense can be isolated within plant tissues disinfected surface, causing damage to the host [7].

Several studies have shown the potential of endophytic bacteria for biological control of diseases, pests, and in promoting the growth of the host plant, being beneficial to the plant. In view of the favorable conditions to sugar cane production, the use of diazotrophic bacteria combined with technologies such as pre-sprouted seedlings production are possible alternatives to increase the productive potential of the Tocantins.

II. MATERIALS AND METHODS

The experiment was conducted in the experimental area of the University Campus of Gurupi from the Federal University of Tocantins, located 280 m altitude, 11º43'S e 49º04'W, where were grown seedlings. We have selected three varieties of sugar cane, and varieties of the early cycle: CVSP077231, a median: CTC4, and a belated: IACSP955000.

At the end of the month of January 2015, the sugarcane separated and the preparation of nodal segments wheels was done according to the methodology presented by [4]. First, it was made the cut of the cane in 3 cm segments containing the node in a hand-crafted guillotine that allowed the cut in a standard size. These nodal segments have undergone heat treatment, subject to water, with constant temperature of 50 C for a period of 30 minutes. After the heat treatment, the nodal segments were placed in trays of sprouting, being separated by cultivating lined up and over to the top, where it contained as substrate: a bucket and a half of commercial substrate Bioflora®, half a bucket of sand and kept in a greenhouse in a period of 10 days, when he had already finished the sprouting.

Twenty days after sprouting the seedlings were transplanted to the cells where the half was planted in cells that had as soil substrate of gully Jatropha pie and the other half in cells just with the soil of gully. Resulting in a total of 275 cells. Data were collected as the greater length and diameter of the stem and soil samples to analyze the fertility of both substrates.

After the transplanting of seedlings of sugarcane to the cells has been prepared the bacteria solution $9{,}33x10^7$ ufc.ml were applied to seedlings, 5 ml of bacteria per cartridge, using an automatic pipette. 10 days after transplanting of seedlings to the cells the first pruning in all the seedlings and was done on a weekly basis due to your accelerated growth. The monitoring of seedlings development weekly.

It required the application of commercial fertilizer, MatoVerde® 15-15-20, in the proportion of 14 g of the product in 7 L of water to 25 ml in each cell, applied every two weeks and then monthly, with the aid of a 30 ml syringe.

Five months after transplanting was accomplished the collection of plants, separating them in the root system and aerial. The shoot was dried in an oven of forced circulation the 60 °C until constant weight and then the heavy semianalítica balance was. The roots were washed under running water with care to avoid losses and after washing, they were also dried in an oven of forced circulation the 60 °C until constant weight, for determining dry mass.

The data of the variables of dry pasta from the shoot and root dry mass were analyzed with the help of the Sisvar program, through the Tukey test, the 5% probability.

III. RESULTS AND DISCUSSION

Generally, the difference was found for root dry mass in relation to the presence of the bacterium; and for dry pasta from the shoot to the substrate type (Table 1).

To the implementation conditions of this study, the results of the combined analysis of variance (Table 1) not show significance ($p < 0.05$) for the triple interaction of the factors' substrate x cultivars x bacteria (BAC x SUB x CULT) for the characters evaluated, indicating that the bacteria interacted with the substrate in root dry mass and substrate interacted with the cultivars for the same variable.

Table1. Summary of the analysis of variance. ADM (Aerial Dry Mass) and RDM (Root Dry Mass) of cultivars of sugarcane in relation to presence of bacteria in the soil substrates of gully and gully solo more Jatropha pie.

Cause of variation	**GL**	**Mean Square**	
		ADM	**RDM**
Bacteria (BAC)	1	0,0002^{ns}	0,0655*
Substrate (SUB)	1	1,2117**	0,0065^{ns}
Cultivar (CULT)	2	0,0983^{ns}	0,0038^{ns}
BAC*SUB	1	0,0085^{ns}	0,0181^{ns}
BAC*CULT	2	0,0696^{ns}	0,0411*
SUB*CULT	2	0,1158^{ns}	0,0462*
BAC*SUB*CULT	2	0,0177^{ns}	0,0106^{ns}
Error	88	0,0526	0,0125
Average	----	0,9811	0,3835
CV (%)	----	23,38	29,18

* and ** significant at the level of 5 and 1% probability of error by Tukey test, respectively; NS not significant at the 5% level of error probability by Tukey test.

No statistical difference was observed between cultivars; even these being of different cycles, aprecocious, median and late(table1).

Table2. Average test to the dry mass of the shoot (ADM) seedling of sugar cane in the land for construction and ground substrates for construction more Jatropha pie.

Substrate	**Average**
Jatropha pie	1,0870 a
Soil of Gully	0,8751 b

Medium followed by the same letter doesn't differ by Tukey test at 5% probability.

For the substrate difference to the dry mass of the aerial part already in the root, there is no difference between the substrates (table 1), where the plants were grown with the substrate soil of gully had the higher fresh mass of the shoot (table 2). [8] evaluating different substrates is a difference for the parameters of above ground (leaves, diameter, and height) of sugarcane of early and medium-late cycle corroborating with the research data confirming that the growth of sugarcane suffers influences of a substrate.

For root dry mass, cultivars IACSP955000 showed no difference with the presence of the bacteria, perhaps because it is a cultivar of the late cycle requires a longer time to demonstrate the effect. The cultivars CVSP077231 e CTC4 presented larger in relation to average values without inoculation, showing that there.

*Table.3. Average values for the interaction and Grow Bacteria (BAC * CULT) to root dry mass (MDR), in seedlings of sugarcane.*

Cultivars			
Bacteria	**(IACSP955000)**	**(CVSP077231)**	**(CTC4)**
Without	0,3661 aA	0,3450 bA	0,3356 bA
With	0,3983 aA	0,4439 aA	0,4122 aA

Medium followed by the same letter, lowercase letters in columns and capitals do not differ by Tukey test at 5% probability.

The difference for each of the cultivars with and without bacteria can give by symbiosis established between them, thus showing the effect on two of them.

The canes grown on substrate Jatropha pie did not differ statistically between them- selves, and in soil of gully the best result was to cultivate CVSP077231, and the other did not demonstrate statistical difference among themselves (Table 4), perhaps because it is a cultivar of early cycle, growing faster than the other.

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*Table4: Average values for the Substrate interaction and Growing (SUB * CULT) to root dry mass (RDM), in seedlings of sugarcane.*

	Cultivares		
Substrate	**IACSP955000**	**CVSP077231**	**CTC4**
Soil of Gully	0,3739 aB	0,4228 aA	0,3306 bB
Jatropha pie	0,3906 aA	0,3661 aA	0,4172 aA

Medium followed by the same letter, lowercase letters in columns and capitals; do not differ by Tukey test at 5% probability.

Jatropha pie consists of up to 69.7% of the mass of seeds and is a by-product of oil extraction composed of cellulose, hemicellulose, lignin, extractive s, water, nutrients, forbol and curcina esters with properties compared to other fertilizers organic, highlighting the amount of soluble in neutral detergent fiber (NDF), acid detergent soluble fiber (FDA), cellulose and crude protein [9], being because of those factors that it was better to the culture and specifically for cultivating CTC4, acting as a source of organic matter for the plant to more than just in soil of gully.

The bacterium promoted initial root system effect, perhaps due to greater endophytic colonization initially in the roots, as this just bacterium infects roots, stalks and leaves of grasses, not being found in leaves of sugar cane [10]. However, the mechanisms of these beneficial associations between plants and the bacterium Herbaspirillum frisingense are still little known. Working with different diazotrophic bacteria, [11], stated that the positive interaction of the bacteria with the root growth of Brachiaria brizantha plants that can contribute to the improvement of the global acquisition of nutrients and water to the plant and the production of biomass.

And that the diazotrophic bacteria colonizers, as h. seropedicae, promotes root growth through various factors such as greater enzyme nitrogenase activity (ARA), phosphate solubilization, hormone production 3-indole-3-acetic acid (AIA), among others as confirmed by [11]. It is known that this hormone has influence on plant growth, especially in the root system [12] which explains the greater root dry mass in plants inoculated with H. frisingense (table 1).

IV. CONCLUSION

There is a beneficial effect on the use of *Herbaspirillum frisingense* bacteria in sugar cane, promoting changes in plant growth, mainly in root development, independent of cultivar tested.

The substrate of Jatropha pie turned out better for the cultivars in relation to the soil of gully, due to have higher concentration of nutrients, providing better conditions for the development of plants.

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